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Population dynamics of *Cacopsylla melanoneura* (Hemiptera: Psyllidae) in Northeast Italy and its role in the Apple proliferation epidemiology in apple orchards

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Abstract

In the present study, incidence of '*Candidatus* Phytoplasma mali' in an experimental apple orchard in Northeast Italy, in addition to abundance and phytoplasma infectivity of *Cacopsylla melanoneura* (Hemiptera: Psyllidae) were determined and the role of this psyllid as a vector of '*Ca.* P. mali' in this region were reviewed. Insect samples collected in the orchard by the beating method indicated high abundance of *C. melanoneura*, (up to 7.92 specimens/branch); however the psyllid *C. picta* was not observed. Molecular analyses revealed presence of '*Ca.* P. mali' in 6.25% of overwintered psyllids. This infection rate is quite high in comparison to other localities where *C. melanoneura* is known as the main vector of the phytoplasma. This finding supports the assumption that *C. melanoneura* also is paramount in the epidemiology of the apple proliferation disease also in Northeast Italy. Moreover, we correlated immigration dynamics to the temperatures registered in the apple orchard, and defined an immigration index to predict the progressive arrival of the overwintered adults from winter sites. Psyllids start to reach the apple orchards when either the average of the maximum temperature of the seven days is above 9.5°C or the immigration index has a positive value. This index will be a useful tool for the growers to prevent apple proliferation phytoplasma spread with well-timed insecticide treatments targeted against *C. melanoneura*. However, further research is needed to validate or adjust the index to other apple growing regions, which may affect more efficacious management of this disease and psyllid vector.

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KEY WORDS: *psyllid vector, '*Candidatus *Phytoplasma mali', immigration dynamics, temperature, apple orchards, overwintered adults,*

Introduction

Apple proliferation is a serious disease which has caused significant economic losses over the past ten years in all European apple-growing regions (Kunze 1989). Fruit weight is often reduced by 30-60%, the fruit color is unsatisfactory and the taste is poor, with the result that as much as 80% of the fruits is unmarketable.

The disease is induced by '*Candidatus* Phytoplasma mali', a phytoplasma that colonizes the phloem system of apple plants and is transmitted by insect vectors during their trophic activity. The pathogen is acquired passively during feeding in the phloem of infected plants, moves through the intestine and pass intracellularly through the epithelial cells to enter the hemocoel. Then the phytoplasma circulates in the hemolymph, replicate and finally penetrates specific cells of the salivary glands. With the next feeding, the insect can transmit the phytoplasma to a healthy plant. Affected plants lack vigor and fruits are markedly reduced in size with poor flavor and low sugar and acidity content (Osler and Loi 1986). Since the first incidence occurred in the Trentino region (northeast Italy) in the mid-1990s, many studies were carried out concerning the spread of the phytoplasma and the insect vectors. High percentages of symptomatic plants were first observed in several orchards of Trentino (Vindimian and Delaiti 1996, Vindimian et al. 2000) affecting several varieties, mainly Golden Delicious, Florina and Renetta del Canada. Studies on insect vectors revealed the important role of the Hemiptera, genus *Cacopsylla* in the transmission of this phytoplasma. In Trentino two species, *Cacopsylla melanoneura* (Förster) and *C. picta* (Förster), are regularly present in the orchards. Contrary to Northwestern Italy where *C. melanoneura* is the primary vector of 'Ca. P. mali' (Tedeschi et al. 2002, 2003; Tedeschi and Alma 2004), in Trentino low phytoplasma infection rate in this species and very low transmission efficiency by this psyllid were recorded (Mattedi et al. 2007, 2008). But in a recent

81 report elevated phytoplasma infection rates in *C. melanoneura* from Trentino region were
82 correlated with the infection level in the plants (Malagnini et al. 2010). On the other hand, *C.*
83 *picta* showed higher natural infection rate and transmission efficiency even at low density
84 (Frisinghelli et al. 2000, Forno et al. 2002, Mattedi et al. 2007, 2008) in accordance also with
85 German results (Jarausch et al. 2003, 2004, 2008, Mayer et al. 2009). Both these insects have a
86 quite complex life cycle. The overwintered adults reach the apple orchards in winter, in the case
87 of *C. melanoneura* (Tedeschi et al. 2002; Mattedi et al. 2007) and at the end of March in the case
88 of *C. picta* (Mattedi et al. 2007). On this host plant they mate, lay eggs and develop. In late
89 spring the newly emerged adults rapidly move to shelter plants, mainly conifers, for aestivation
90 and overwintering.
91 Preliminary evidence suggests that overwintered adults are already infected when they re-
92 migrate into apple orchards (Jarausch et al. 2004; Mattedi et al. 2008), so well-timed treatments
93 are very important to control the first individuals that reach the orchards. For this reason a
94 phenology model will be a useful support for vector management decisions. Many forecasting
95 models have been produced for other psyllid species, using means of driving variables, mainly
96 temperature, and based on developmental thresholds (DT) and degree-days (DD) (Beránková and
97 Kocourek 1994, Kapatos and Stratopoulou 1999, Kumral et al. 2008, Morgan and Solomon
98 1993; Schaub et al. 2005). These models refer to some phases of the life cycle and predict when
99 a developmental stage (generally the most damaging stage) will appear. None of these models
100 concerns the immigration period for psyllids that move between different host-plant species or
101 exploit non-host plant species as overwintering sites.
102 In the present study we analyzed in detail the incidence of '*Ca* P. mali' in an experimental
103 orchards in Northeast Italy in addition to the abundance and phytoplasma infection of *C.*

104 *melanoneura*, with the aim to revise the role of this psyllid as a vector of '*Ca* P. mali' in this
105 region. Moreover, we propose an index based on the maximum temperatures registered in the
106 apple orchard, to predict the arrival of the overwintered adults. This index will be a useful
107 management tool for the growers to prevent phytoplasma spread thanks to well-timed insecticide
108 treatments.
109
110 ## Materials and methods
111 **Field samplings**
112 The study was carried out over five growing seasons (2006-2010) in a conventionally treated 3
113 hectare apple orchard of the IASMA Research and Innovation Center, Fondazione E.Mach
114 (Borgo Valsugana, 419 m a.s.l., Trento, Italy). Observations on the incidence of Apple
115 proliferation disease and the population dynamics of *C. melanoneura* were performed in 4
116 untreated plots (area 1500 m²), which were randomly selected in the orchard. The main apple
117 variety was Golden delicious, with 15-25 years old trees, 4.0±0.5 m high, and spaced 0,5-1.6 m
118 within a row and 4.0-4.6 m between rows. The number of symptomatic trees was recorded at the
119 end of September and a total number of twenty-one samples was randomly collected from
120 symptomatic plants to be analyzed with molecular assays. During each season, insect monitoring
121 started before the immigration in the orchard of the first overwintering adults (January/February)
122 and it lasted till the emergence of the springtime generation (May/June). Adults were collected
123 every 7 days, by means of beating method: for each replication plot, 25 branches (50±10 cm in
124 length) were considered and every branch was shaken 2 times above a beating tray (diameter 7
125 cm, 60 x 40 cm of cloth). The collected adults were counted and identified in the laboratory by
126 both morphological, examining female and male terminalia (Ossiannilsson, 1992) and molecular

127 tools (Tedeschi and Nardi 2010), then analyzed for phytoplasma presence. An in-depth study on
128 the frequency of '*Ca.* Phytoplasma mali'-positive psyllids was carried out in 2008 and a total
129 number of 194 batches, each of 5 specimens, was analyzed. The number of eggs and young
130 stages was assessed by examining under the microscope 30 apical shoots (20-25 cm long) from
131 each replication, randomly selected in the central rows of each plot. These controls were
132 performed every 7 days, from the beginning of the oviposition until the emergence of the
133 springtime generation.
134
135 **Phytoplasma detection and species identification**
136 Plant DNA was isolated from 100 mg (wet weight) of phloem tissue from symptomatic plants,
137 previously ground with liquid nitrogen in a sterile mortar, using the QIAGEN's DNeasy® Plant
138 Mini Kit (Qiagen, Hilden, Germany). DNA was eluted in 100 µl of elution buffer and kept at -
139 20°C until used. Total DNA was extracted from batches of five adult psyllids following a
140 protocol adapted from Marzachì et al. (1998) and previously applied to psyllids (Tedeschi et al.
141 2002). The final product was resuspended in 50 µl of TE.
142 Insect and plant DNAs were amplified firstly with the phytoplasma universal primer pair P1/P7
143 (Schneider et al. 1995) and then in nested PCR with the primers fO1/rO1 (Lorenz et al. 1995)
144 specific for the AP-group phytoplasmas, after a 1:40 dilution. Reaction conditions were as in the
145 original papers. Contamination by amplicons was avoided by using separate rooms and material
146 as well as decontamination procedures (UV exposure and bleaching of materials and surfaces).
147 Moreover, for each amplification, a negative control containing Milli-Q water was
148 included.Amplification products were analyzed by 1% agarose gel electrophoresis, stained with
149 ethidium bromide, and visualized on a U.V. transilluminator. Specific '*Ca.* Phytoplasma mali'

150 profiles were obtained by RFLP analysis with endonuclease SspI, digesting seven microliters of
151 the amplification product with 3U of SspI for 4.5 h at 37°C.
152 The proportion of infected insects was estimated by its maximum-likelihood estimator, ^p,
153 calculated according with Swallow (1985): $\hat{p} = 1-H^{1/k}$, where H is the observed fraction of
154 healthy groups and k is the number of insects per group, five in this case.
155 The psyllid identification by morphological analyses was confirmed by molecular analyses using
156 the primer pairs MEL fw/MEL rev developed for *C. melanoneura* (Tedeschi and Nardi 2010).
157 Thirty-two randomly selected individuals were used to confirm morphological observations.
158 Reaction conditions were as in the original papers.
159
160 **Population dynamics and index of immigration**
161 The population trend of *C. melanoneura* in the apple orchards was based on data collected during
162 the years 2006-2010 for adults and 2006-2008 for eggs and juveniles. In order to identify which
163 environmental parameters in the orchards were possibly involved in *C. melanoneura*
164 colonization we developed an original model according to the following procedure. Starting,
165 from 2006 the maximum, and median daily and hourly temperatures were recorded at 2 meters
166 above the ground from a meteorological station inside the apple orchard. For daily temperatures
167 we also calculated the average of the seven days ($\mathbf{T_{7n}}$) preceding any sampling date ($\mathbf{a_n}$). Seven
168 days was chosen since field samplings were conducted with such a periodicity. Temperature
169 trends were associated with the insect dynamics, from the date of first detection ($\mathbf{a_0}$) to the peak
170 of captures ($\mathbf{a_{max}}$). In the first instance, the absolute highest temperature among the 7 days
171 preceding a_0 ($\mathbf{T_{0max}}$) was taken and then the minimum across the years among the T_{0max}. This
172 minimum was defined as hypothethical threshold temperature (**Tth**) of psylla orchard

immigration. The Tth, calculated both for median and maximal temperatures was checked whether it had been previously passed without eliciting the psyllid immigration and we selected only those that were not passed. The same procedure was done for the T_{7n} where a relative hypothetic threshold (**T_7th**) was defined.

The average of degree days were counted with temperature above the threshold for any T_{7n} (**dd_n**).

The index of immigration was defined as:

Ii = [$(T_{7n} - T_7th) + dd_n$]

The immigrated population (**Ip**) as:

$(p_n - p_{n-1})$

where $(p_n - p_{n-1})$ is the difference of sampled adults (expressed as % referred to the population peak) between two consecutive samplings.

A simple regression analysis was performed between **Ii** and **Ip**, from the a_0 to the a_{max} of each year (2006-2010). If the regression was significant then the previsions were verified concerning the detection of any a_0 by calculating **Ii** related to any sampling from 2006 to 2010 *a posteriori*. The first positive value of Ii was assessed whether it was associated to the first psylla detection in the field. The same procedure was followed for historical data taken from the same orchard in the period 2002-2004. Eventually, all data were associated to the apple phenology, in particular bud breaking and first flowering.

Results

Apple proliferation incidence

195 The symptoms observed in the orchards were predominantly witches' brooms, reddening of the
196 leaves, enlarged stipulae, abnormally long flower stalks, flowering out of season and small fruits.
197 Basing on the percentage of symptomatic trees, the incidence of apple proliferation disease
198 ranged from 60 to 100%, according to the plot, with a mean annual increase of 5%.
199
200 **Psyllid identification and phytoplasma detection**
201 Following the morphological observations, almost all the psyllids collected in the experimental
202 plots were identified as *C. melanoneura*. *C. mali* was sporadically observed; whereas, *C. picta*
203 was never found. Molecular analyses confirmed the identifications of *C. melanoneura*.
204
205 The PCR analyses performed with primer pair fO1/rO1 gave AP-group specific amplicons of the
206 expected size (1050 bp) with both plant and insect DNA. No amplification products were
207 obtained from the negative controls. All 21 samples of symptomatic plants were positive to AP-
208 group phytoplasmas and the RFLP with *Ssp*I restriction enzyme confirmed the presence of '*Ca.*
209 Phytoplasma mali'. The results of PCR detection in the insects are shown in table 1. One
210 hundred and seventy-four samples of overwintered adults, 5 samples of nymphs and 15 samples
211 of newly emerged adults were tested. Forty-eight out of 174 batches of overwintered psyllids
212 were positive for '*Ca.* Phytoplasma mali', with an estimation of 6.25% of infected *C.*
213 *melanoneura.* No amplification was obtained from nymphs and newly emerged adults.
214
215 **Population dynamics and index of immigration**
216 The population trend of *C. melanoneura* overwintered adults was extremely variable during the
217 years . The immigrant adults were recorded starting from the beginning of February in 2008,

218 while in 2006 they were delayed to mid-March. The peak of overwintered adults was reported
219 between the 10th and the 13th week, depending of the year, but it was mostly concentrated in the
220 second half of March. Eggs of *C. melanoneura* were observed on apple branches starting from
221 the beginning of March in 2008 and the last ten days of the month in 2006 and 2007. The
222 oviposition lasted until mid-April in 2007 and the beginning of May in 2006 and 2008. Young
223 stages were recorded from the beginning of April in 2007 and 2008 and the end of the same
224 month in 2006. Nymphs remained in the orchards until the beginning of May in 2007 and the end
225 of May in 2006 and 2008 (Fig. 1).
226 Combining the population dynamics of *C. melanoneura* with the apple phenology (Table 2), a
227 good correspondence between overwintered adult peak – first egg detection and apple bud
228 breaking was found, with the peak of egg always preceding the first flowering.
229 Values and indexes referred to the psylla migration analysis are reported in table 3. The **Tth**
230 calculated from maximum (12.8°C) and median (3.3°C) temperatures did not explain the first
231 appearance of the psylla in the orchard, and no correlation was found with the insect migration
232 into the orchard. Similarly, the **T_7th** taken from median temperatures (2.0°C) did not represent
233 an effective threshold value, because it has been passed several times in the years without
234 triggering the appearance of psylla in the orchard. Instead, the **T_7th** calculated from maximum
235 values (9.5°C) was found to be a candidate for a possible migration threshold. The regression
236 analysis between **Ip** and **Ii** (Fig.2) was highly significant ($F = 24.2$; $df = 1,26$; $p < 0.001$).
237 Positive values of **Ii** corresponded with the first appearance of the psylla in the orchard in 2006
238 and 2008-10. Instead, in 2007 values slightly above zero (1.5 and 0.9) in the first five weeks did
239 not correspond to the first presence. However, when the **Ii** reached for a more relevant value

240 (6.5), individuals were eventually found. Positive **Ii** fitted in verifying all the $\mathbf{a_0}$, also for
241 historical data of 2002-2004.
242
243 ## Discussion
244 The present research provides important and new information on the epidemiology of apple
245 proliferation in northeast Italy and develops an index of immigration that enables well-timed
246 control measures against the insect vectors. The role of *C. melanoneura* as vector of '*Ca.*
247 Phytoplasma mali' has been revised in this area confirming the results obtained by Malagnini et
248 al. (2010) on psyllid infectivity. Previous reports concerning Trentino region pointed out the
249 predominance of *C. picta* in Val d'Adige and *C. melanoneura* in Val di Sole and Val di Non (up
250 to 2 overwintered adults/branch) (Mattedi et al. 2007, 2008). Transmission trials and
251 phytoplasma quantification in insect bodies revealed a low transmission efficiency for *C.*
252 *melanoneura* while an important increase in phytoplasma concentration was observed only in *C.*
253 *picta*, indicating an efficient phytoplasma multiplication in this species (Pedrazzoli et al. 2007).
254 For this reason it is now popularly held belief that *C. picta* is the most important vector of '*Ca.*
255 Phytoplasma mali' in northeast Italy, as well as in Germany (Jarausch et al. 2003, 2004, 2008;
256 Mayer et al. 2009), while in northwestern Italy, *C. melanoneura* represents the only psyllid
257 vector of this phytoplasma (due also to the absence of *C. picta*).
258 Evidence supports the role of *C. melanoneura* as a vector in Valsugana, a valley in the south east
259 of the Trentino region with a long tradition in apple production. This area is characterized by an
260 high incidence of the apple proliferation disease and the only important psyllid species which
261 was found in the repeated collections trough a five year period was *C. melanoneura*; *C. picta* was
262 never recorded. The molecular analyses revealed the presence of '*Ca.* Phytoplasma mali' (as the

263 Swallow's estimated proportion p^) in 6.25% of overwintered psyllids, confirming roughly the
264 results obtained by Malagnini et al. (2010) with specimens coming from the same area. This
265 discrepancy may be due to the different number of psyllids analyzed. During the peak presence
266 there were up to 7.92 overwintered adults/branch in 2007, and from the beginning until the end
267 of March in all the three years the number of recorded *C. melanoneura* was almost always higher
268 than 2 psyllids/branch. Thus, in this valley, *C. melanoneura* should be considered the only
269 significant vector associated with the rapid spread of this disease.
270 The infection rate of the overwintered adults tested high in comparison to other localities where
271 *C. melanoneura* is already known as the main vector of '*Ca.* phytoplasma mali' (an estimation of
272 6.25% vs 3.6% of '*Ca.* Phytoplasma mali'-positive insects) (Tedeschi et al. 2003). This fact
273 strengthens the evidence that *C. melanoneura* has an important role in the epidemiology of the
274 apple proliferation disease also in northeast Italy, where its control is already regulated by
275 insecticide treatments (Baldessari et al. 2007, 2009).
276 Once ascertained that *C. melanoneura* represents an important risk for apple growers, a new
277 approach to the management of this vector needs to be developed. Data collected during the
278 years concerning the population dynamics of this species, the apple phenology and the weather
279 parameters allowed us to define an index to predict the re-migration of overwintered adults into
280 apple orchards. The aim was to provide to the growers an easy to use tool which would predict
281 the time of arrival of *C. meanoneura*.in order to refine the pest management decisions. The
282 efficiency of an insecticide treatment depends on the product and on the timing of application in
283 order to affect the most sensitive or harmful stage on the crop. In the case of *C. melanoneura*,
284 overwintered adults showed, in comparison with all the other stages, the highest population
285 density, the highest percentage of '*Ca.* Phytoplasma mali'-positive specimens, and the longest

286 time spent in apple orchards. All these characteristics suggest that this is the crucial role of this
287 stage in vectoring AP-phytoplasma (Tedeschi et al. 2002, 2003). For this reason the treatments
288 should be focused on the overwintered adults as soon as they start to colonize apple orchards.
289 As a consequence, we established a index of immigration related to the immigrant psylla adults.
290 Similar studies were built in the past to forecast the occurrence of a particular life stage of a pest
291 with the aim to improve its control (Beránková and Kocourek 1994, Kapatos and Stratopoulou
292 1999, Kumral et al. 2008, Morgan and Solomon 1993, Schaub et al. 2005), but never to predict
293 the adult immigration in the orchards. Here we propose an empirical model which cannot be
294 supported by laboratory trials due to the impossibility of reproducing the entire biological cycle
295 of *C. melanoneura* in controlled conditions. This correlation is based on the temperatures
296 recorded in the apple orchards, a variable that can be easily monitored by the growers or at least
297 by the phytosanitary services.
298 It is known that temperature influences apple phenology and that the migration could also be
299 influenced by certain phenological stages of the trees. Immigration into orchards take place
300 during budding, which may be detectable by the overwintering psylla, thereby attracting them to
301 the stimuli associated to budding.
302 However, the distances occurring between psyllid host plants and shelter plants can be
303 considerable (Čermák and Lauterer 2008, Thebaud et al. 2009), thus it is improbable and
304 temperature is most likely thethe critical factor. There was no correlation between immigration
305 dynamics and apple phenology demonstrated, however oviposition occurs at bud burst while egg
306 peak and hatchings are always before the first flowering. This confirmed a good degree of
307 synchrony between *C. melanoneura* and host-plant growth, being linked with temperatures as

308 stated for psyllids in general by Hodkinson (2009). These data are likewise useful for the
309 growers for possible further treatments during the season.
310 In the present study, the hypothetic threshold calculated as an average of the maximum
311 temperatures of the seven days (**T_7th**) proved to be a good tool to forecast immigration, by
312 calculating a proper Index of immigration (**Ii**). Psyllids started to reach the apple orchards when
313 the **T_7th** was 9.5°C. Also including in the model single episodes, meant as hours above this
314 threshold, we could individuate with extremely precision the time of first occurrence and the
315 following immigration trend for a period of 9 years. It is feasible that any **Ii** over 0 can elicit
316 psyllid migration, with direct correlation between **Ii** value and number of migrant specimens, and
317 our failure in detecting individuals in correspondence of **Ii** < 2 depended likely on the extremely
318 low number occurring in the field in those circumstances.
319 On the other hand, there are still some relevant limits that we need to point out. The calculated
320 threshold fits well for apple orchards located in the Valsugana valley, but not necessarily for
321 other locations, where the correlation needs to be validated by adjusting the threshold value,
322 either according to historical collection data or by programming periodical field collections.
323 Other geographical factors associated with the winter sites location (e.g. the regional orography,
324 the main air streams and distance from apple orchards) may differently affect the psylla
325 migration process and influence its presence/absence, both in terms of time and quantity, in a
326 given apple orchard.
327 A straight correlation between maximum temperature and adult mobility was found in addition
328 to a, temperature threshold that, independently on the period in the winter, favors the psylla to
329 abandon overwintering sites. However, more research is required to set up a proper forecasting
330 model, which could be applied in different apple-growing regions.

References

Baldessari, M., F. Trona and G. Angeli. 2007. Evaluation of different insecticides and strategies against *Cacopsylla melanoneura* (Förster) (Homoptera: Psyllidae), a vector of apple proliferation phytoplasma. Commun. Agric. Appl. Biol. Sci. 72: 423-429.

Baldessari, M., F. Trona, G. Angeli and C. Ioriatti. 2010. Effectiveness of five insecticides for the control of adults and young stages of *Cacopsylla melanoneura* (Förster) (Hemiptera: Psyllidae) in a semi-field trial. Pest Manage. Sci. 66: 308-312.

Beránková, J. and F. Kocourek. 1994. The monitoring of the phenology and population dynamics of the pear psylla (*Psylla pyri* L.). Ochr. Rostl. 30: 283-292

Čermak, V. and P. Lauterer. 2008. Overwintering of psyllids in South Moravia (Czech Republic) with respect to the vectors of the apple proliferation cluster phytoplasmas. B. Insectol. 61: 147-148.

Forno, F., A. Branz, L. Mattedi, P. Bragagna, C. Cainelli, D. Forti, and M.E. Vindimian. 2002. Un triennio di prove di trasmissione di AP tramite psille. *In* ATTI Giornate Fitopatologiche, Baselga di Pinè (Trento) 7-11 April 2002, vol 2: 613-616.

Frisinghelli, C., L. Delaiti, M.S. Grando, D. Forti and M.E. Vindimian. 2000. *Cacopsylla costalis* (Flor 1861), as a vector of apple proliferation in Trentino. J. Phytopathol. 148: 425-431.

Hodkinson, I. 2009. Life cycle variation and adaptation in jumping plant lice (Insecta: Hemiptera: Psylloidea): a global synthesis. J. Nat. Hist. 43: 65-179.

Jarausch, B., N. Schwind, W. Jarausch, G. Krczal, E. Dickler and E. Seemüller. 2003. First report of *Cacopsylla picta* as a vector of apple proliferation phytoplasma in Germany. Plant Dis. 87: 101.

Jarausch, B., N. Schwind, W. Jarausch and G. Krczal. 2004. Overwintering adults and springtime generation of *Cacopsylla picta* (synonym *C. costalis*) can transmit apple proliferation phytoplasmas. Acta Hort. 657: 409-413.

Jarausch, B., A. Fuchs, N. Schwind, and W. Jarausch. 2008. Efficienza di trasmissione delle psille: l'esperienza in Germania, pp. 127-135. *In* C. Ioriatti and W. Jarausch (eds.), Scopazzi del melo Apple Proliferation. Fondazione Edmund Mach, San Michele all'Adige,TN, Italy.

Kapatos, E.T. and E.T. Stratopoulou. 1999. Duration times of the immature stages of *Cacopsylla pyri* L. (Hom., Psyllidae), estimated under field conditions, and their relationship to ambient temperature. J. of Appl. Entomol. 123: 555-559.

Kumral, N., B. Kovanci and B. Akbudak. 2008. Using degree-day accumulations and host phenology for predicting larval emergence patterns of the olive psyllid, *Euphyllura phillyreae*. J Pest Sci. 81: 63-69.

Kunze, L. 1989. Apple Proliferation, pp. 99-113. *In* P.R. Fridlund (ed.), Virus and Viruslike Diseases of Pome Fruits and Simulating Noninfectious Disorders. Cooperative Extension College of Agriculture and Home Economics, Washington State University, Pullmann, Washington.

Lorenz, K.H., B. Schneider, U. Ahrens, and E. Seemüller. 1995. Detection of the apple proliferation and pear decline phytoplasmas by PCR amplification of ribosomal and nonribosomal DNA. Phytopathology 85: 771-776.

Malagnini, V., F. Pedrazzoli, V. Gualandri, R. Zasso, E. Bozza, F. Fiamingo, A. Pozzebon, N. Mori, and C. Ioriatti. 2010. Detection of '*Candidatus* Phytoplasma mali' in different populations of *Cacopsylla melanoneura* in Italy. B. Insectol. 63: 59-63.

376 **Marzachì, C., F. Veratti, and D. Bosco. 1998.** Direct PCR detection of phytoplasmas in
377 experimentally infected insects. Ann. of Appl. Biol. 133: 45-54.
378 **Mattedi, L., F. Forno, and M. Varner. 2007.** *In* Scopazzi del melo. Conoscenze ed
379 osservazioni di campo. Arti Grafiche La Commerciale-Borgogno, Bolzano, Italy.
380 **Mattedi, L., F. Forno, C. Cainelli, M.S. Grando, and W. Jarausch. 2008.** Research on
381 *Candidatus* Phytoplasma mali transmission by insect vectors in Trentino. Acta Hort. 781:
382 369-374.
383 **Mayer, C.J., B. Jarausch, W. Jarausch, W. Jelkmann, A. Vilcinskas, and J. Gross. 2009.**
384 *Cacopsylla melanoneura* Has No Relevance as Vector of Apple Proliferation in Germany.
385 Phytopathology 99: 729-738.
386 **Morgan, D. and M.G. Solomon. 1993.** PEST-MAN: a forecasting system for apple and pear
387 pests. 23: 601-605.
388 **Osler, R. and N. Loi. 1986.** Apple proliferation disease, pp. 59-73. Proceedings, U.S. – Italian
389 Workshop on Fastidious prokariotes: cultivation, detection, and associated economic
390 problems. 2-4 June 1986, New Brunswick, New Jersey...
391 **Ossiannilsson, F. 1992.** The Psylloidea (Homoptera) of Fennoscandia and Denmark. Fauna
392 Entomologica Scandinavica 26. E. J. Brill, Leiden, The Netherlands.
393 **Pedrazzoli, F., V. Gualandri, F. Forno, L. Mattedi, V. Malagnini, A. Salvadori, G. Stoppa,**
394 **and C. Ioriatti. 2007.** Acquisition capacities of the overwintering adults of the psyllid
395 vectors of '*Candidatus* Phytoplasma mali'. B. Insectol. 60 (2): 195-196.
396 **Schaub, L., B. Graf, and A. Butturini. 2005.** Phenological model of pear psylla *Cacopsylla*
397 *pyri*. Entomol. Exp. Appl. 117: 105-111.

Schneider, E., E. Seemüller, C.D. Smart, and B.C. Kirkpatrick. 1995. Phylogenetic classification of plant pathogenic mycoplasma-like organism or phytoplasmas, pp. 369-380. *In* S. Razin and J.G. Tully (eds.) Molecular and Diagnostic Procedures in Mycoplasmology, Vol I,.Academic Press, San Diego,.

Swallow, W.H. 1985. Group-testing for estimating infection-rates and probabilities of disease transmission. Phytopathology 75: 882-889.

Tedeschi, R., and A. Alma. 2004. Transmission of apple proliferation phytoplasma by *Cacopsylla melanoneura* (Homoptera : Psyllidae). J. Econ. Entomol. 97: 8-13.

Tedeschi, R., and F. Nardi. 2010. DNA-based discrimination and frequency of phytoplasma infection in the two hawthorn-feeding species, *Cacopsylla melanoneura* and *Cacopsylla affinis*, in northwestern Italy. Bull. Entomol. Res. 100: 741-747.

Tedeschi, R., D. Bosco, and A. Alma. 2002. Population dynamics of *Cacopsylla melanoneura* (Homoptera : Psyllidae), a vector of apple proliferation phytoplasma in northwestern Italy. J. Econ. Entomol. 95: 544-551.

Tedeschi, R., C. Visentin, A. Alma, and D. Bosco. 2003. Epidemiology of apple proliferation (AP) in northwestern Italy: evaluation of the frequency of AP-positive psyllids in naturally infected populations of *Cacopsylla melanoneura* (Homoptera : Psyllidae). Ann. Appl. Biol. 142: 285-290.

Thébaud, G., M. Yvon, R. Alary, N. Sauvion, and G. Labonne. 2009. Efficient transmission of '*Candidatus* Phytoplasma prunorum' is delayed by eight months due to a long latency in its host-alternating vector. Phytopathology 99 (3): 265-273.

Vindimian, E. and L. Delaiti. 1996. Indagine sistematica sugli scopazzi del melo. Terra trentina 13 (11): 30-33.

421 **Vindimian, M.E., A. Branz, F. Tomasi, and P. Gatto. 2000.** Untersuchungen zum Besenwuchs
422 des Apfelbaumes. Obstbau Weinbau 10: 294-296.

Table 1 - Estimated proportion (p^) of '*Candidatus* Phytoplasma mali'-infected *Cacopsylla melanoneura* (Förster) collected by beat tray samplings in 2008 in the experimental plots

	Date	AP+/tot	p^	AP+/tot	p^
overwintered	1 February	0/3	0	48/174	6.25
	8 February	5/10	12.94		
	15 February	6/8	24.2		
	22 February	1/8	2.63		
	28 February	5/30	3.58		
	7 March	11/30	8.73		
	13 March	8/30	6.01		
	21 March	5/30	3.58		
	28 March	6/20	6.88		
	4 April	1/5	4.36		
nymphs	15 May	0/15	0	0/15	0
offspring	16 April	0/1	0	0/5	0
	15 May	0/4	0		

Table 2 - Number of weeks when an event of psylla or apple phenology was recorded for each year of investigation (2006-2010). For the historical data (2002-2004) only the first detection and the peak of captures of psylla were available.

	2002	2003	2004	2006	2007	2008	2009	2010
First Psylla	5	8	6	11	7	4	8	9
Psylla Peak	10	10	11	13	11	10	12	12
First Egg	-	-	-	13	11	10	12	12
Egg Peak	-	-	-	14	13	14	14	13
Bud break	-	-	-	13	10	11	12	12
Flowering	-	-	-	16	14	14	15	15

Table 3 - Absolute (T) and average of 7 days (T7) median (med) and maximal (max) temperature (°C) associated with the first psylla detection (suffix a_0) and with periods before the first detection (suffix a_{-n}) in the years 2002-2004 and2006-2010 . Ii is the Immigration index. In bold are indicated the minimal a_0 and maximal a_{-n} values across the years for each parameter.

	2002	2003	2004	2006	2007	2008	2009	2010
Ta_{0med}	-	-	-	4.9	7.5	12.9	**3.3**	5.4
Ta_{-nmed}	-	-	-	4.4	**8.5**	4.2	4.5	4.4
Ta_{0max}	-	-	-	12.8	16.4	20.3	**12.5**	13.0
Ta_{-nmax}	-	-	-	11.8	**21.8**	11.4	9.6	9.8
T7$a_{0\ med}$	-	-	-	3.5	5.1	5.3	**2,0**	4.5
T7$a_{-n\ med}$	-	-	-	2.3	**3.7**	3.1	**3.7**	3.0
T7$a_{0\ max}$	9.8	10.2	10.2	10.1	12.1	11.2	**9.5**	10.4
T7a_{-nmax}	5.8	6.6	7.7	8.8	**9.4**	7.0	8.0	7.7

Table 4. Calculated Index of immigration (Ii) and Immigrated population (Ip) from the first week of January until the population peak, in the periods 2002-2004 and 2006-2010. Samplings (s) were conducted weekly. In gray cells, in bold, are highlighted values of first positive Ii and first Psylla detection in the orchard.

	2002		2003		2004		2006		2007		2008		2009		2010	
s	**Ii**	**Ip**	**Ii**	**Ip**	**Ii**	**Ip**	**Ii**	**Ip**	**Ii**	**Ip**	**Ii**	**Ip**	**Ii**	**Ip**	**Ii**	**Ip**
1	-8.0	0	-7.0	0	-7.5	0	-7.0	0	-3,2	0	-7.9	0	-9.5	0	-6.7	0
2	-6.4	0	-9.0	0	-5.2	0	-8.3	0	-0,4	0	-4.8	0	-6.3	0	-6.0	0
3	-6.1	0	-7.0	0	-6.0	0	-8.2	0	**1.5**	0	-2.1	0	-5.6	0	-6.3	0
4	-3.6	0	-2.9	0	-8.2	0	-7.0	0	-3.9	0	**6.1**	**0.03**	-3.8	0	-7.5	0
5	**2.2**	**0.04**	-4.9	0	-1.2	0	-1.7	0	0.9	0	0.0	0.05	-0.9	0	-6.6	0
6	4.3	0.09	-5.0	0	**2.3**	**0.07**	-5.1	0	-1.2	0	1.4	-0.02	-2.6	0	-5.3	0
7	-1.3	-0.09	-1.7	0	2.3	0.11	-2.8	0	6.5	**0.01**	-0.2	0.00	-2.6	0	-3.3	0
8	4.0	0.27	**3.4**	**0.05**	-5.7	-0.04	-3.4	0	7.8	0.01	9.3	0.50	**1.5**	**0.04**	-1.5	0
9	5.1	0.08	6.9	0.50	-0.3	-0.07	-2.5	0	15.3	0.50	12.2	0.26	2.3	0.15	**2.8**	**0.01**
10	14.6	0.61	11.3	0.45	3.2	0.07	-0.1	0	12.2	0.13	4.1	0.19	6.9	0.22	-1.6	0.02
11					6.1	0.85	**3.2**	**0.41**	9.2	0.35			11.4	0.60	6.9	0.17
12							7.8	0.50							15.2	0.81
13							10.8	0.10								

Figure Captions

Fig. 1. Presence of eggs and nymphal stages of *Cacopsylla melanoneura* (Förster) in the orchard in 2006-2008.

Fig. 2. Simple Regression analysis ($F = 24.2$; $df = 1,26$; $p < 0.001$) between Immigration Index (**Ii**) and Immigrated population (**Ip**) calculated from the average of maximal temperature of the 7 days preceding any adult sampling of the period 2002-2010.

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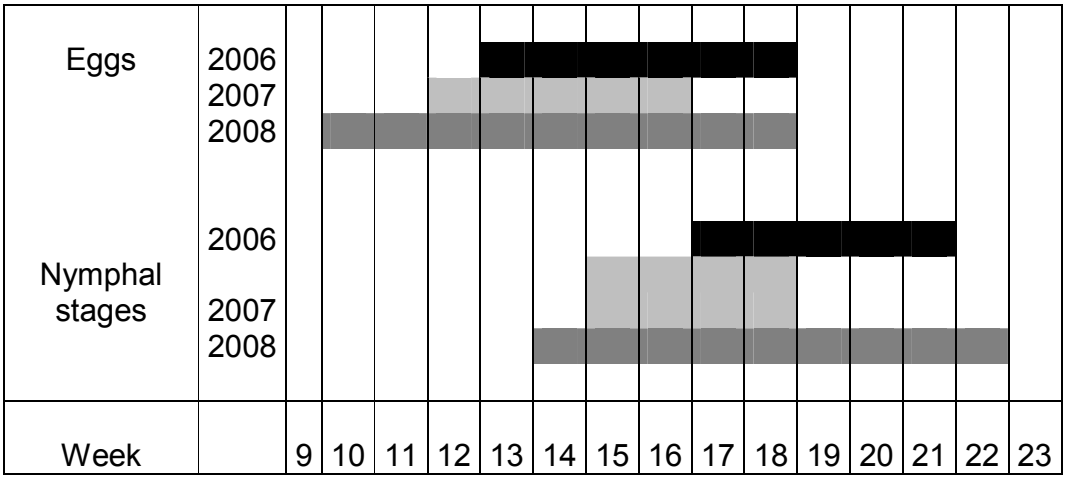

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464 Fig. 1

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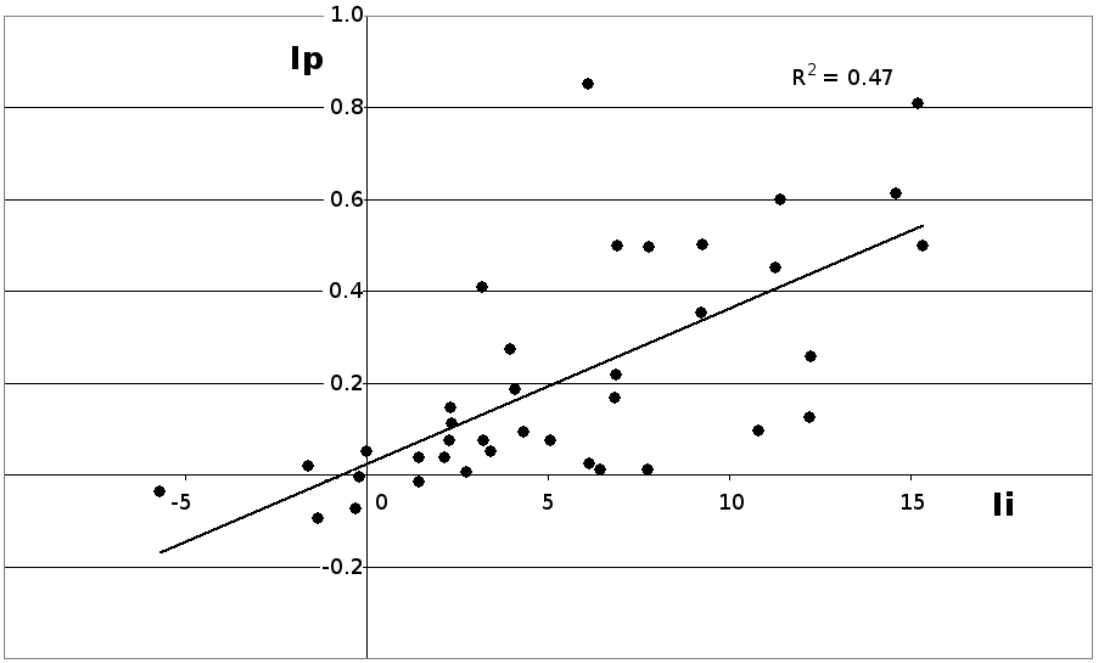

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469 Fig. 2